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In Translation

Memoirs of a Reincarnated T Cell

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In two studies published in this issue of *Cell Stem Cell*, Nishimura et al. (2013) and Vizcardo et al. (2013) reprogram mature, antigen-specific cytotoxic T cells into induced pluripotent stem cells (iPSCs). The antigen-specific iPSCs can be redifferentiated into "rejuvenated" proliferative T cells and have broad applications for adoptive immunotherapy.

One method to confirm the identity of the reincarnated Dalai Lama involves placing several artifacts, only a few of which belonged to the previous Dalai Lama, in front of a candidate child. If the child recognizes an artifact which belonged to the previous Dalai Lama, this is interpreted as a sign that the child is indeed the reincarnated Lama. In the recent work by Nishimura (Nishimura et al., 2013) and Vizcardo (Vizcardo et al., 2013) presented in this issue of *Cell Stem Cell*, we are also witnesses to a majestic reincarnation of sorts. Both groups reprogrammed human antigen-specific $CD8^+$ mature T cells into induced pluripotent stem cells (iPSCs). Furthermore, they showed that redifferentiation of these cells produced $CD8^+$ T cells that recognize their original cognate antigen and have features of rejuvenated cells so critically needed to improve the efficacy of T-cell-based therapies for the treatment of cancer and viral-associated diseases (Fearon, 2007).

Needless to say, the regeneration of antigen-specific T cells from iPSCs derived from mature $CD8^+$ T cells provides yet another example of the reach of Yamanaka's discovery that induction of four transcription factors—Oct4, Sox2, Klf4, and c-Myc—can reprogram well-differentiated somatic cells to pluripotency (Takahashi and Yamanaka, 2006). The field of regenerative medicine and the stem cell community continue to profit from Yamanaka's finding that was fittingly celebrated in Stockholm last month. Amidst immense enthusiasm over the therapeutic potential of iPSCs, however, remain obstacles in translating this discovery to improve the care of patients (Wu and Hochedlinger, 2011). By focusing on the reprogramming of the immune system in adoptive cell transfer immunotherapy, the work of Nishimura et al. and Vizcardo et al. may provide an opportunity to hasten the bench-to-bedside translation of iPSC technology.

Adoptive cell transfer immunotherapy (ACT) of T cells shows great promise in treating patients with advanced cancer, graft-versus-host disease, and viral-associated diseases. As a therapy to treat cancer, for example, ACT relies on the natural ability of $CD8^+$ T cells (called tumor-infiltrating lymphocytes, or TILs) to attack tumors (Restifo et al., 2012). The dogged persistence of cancer, however, often results in $CD8^+$ T cells that are both exhausted and senescent (Klebanoff et al., 2006). With ACT, the tumor-specific TILs are harvested from a patient's tumor, expanded ex vivo with the immunostimulatory cytokine IL-2, and infused back into the same patient (Figure 1). Remarkably, up to 20% of patients with advanced melanoma have had complete and durable regression of their metastatic disease (Rosenberg, 2011). In trying to understand why this cohort of patients had such an extraordinary response, it was found that transfer of less-differentiated memory or stem-cell-like $CD8^+$ T cells correlated with a more robust eradication of tumor. Therefore, there is a critical need to improve the efficacy of ACT by developing methods such as iPSC technology to expand the pool of memory or stem-cell-like $CD8^+$ T cells (Gattinoni et al., 2011).

In their study, Vizcardo et al. attempt to address this need by obtaining TILs from one of our patients suffering from metastatic melanoma at the Surgery Branch of the National Cancer Institute. The TILs have an antigen specificity for MART-1, which is commonly expressed on melanoma tumors. The MART-1-specific TILs were transduced with Yamanaka factors and reprogrammed to iPSCs as evidenced by ESC-like morphology, capacity for teratoma formation, endogenous expression of *OCT3/4*, *SOX2*, *KLF4*, and *c-MYC*, and disappearance of T cell markers CD3 and CD8. Subsequent coculture with Delta-like 1 expressing OP9 feeder cells resulted in redifferentiation to $CD8^+$ T cells (Schmitt and Zúñiga-Pflücker, 2002). When cultured with antigen presenting cells and challenged with the cognate antigen MART-1, reprogrammed cells produced interferon gamma, demonstrating functional integrity.

Although Vizcardo and colleagues show that reincarnated $CD8^+$ T cells have a memory for their original tumor antigen, it is not clear if reincarnated cells are indeed rejuvenated. That is, does the reprogramming process confer greater replicative capacity, multipotency, and antitumor immunity that is characteristic of memory or stem-cell-like T cells? We know that TILs are generally characterized by a state of exhaustion and terminal differentiation. Thus, the lingering question is whether the reincarnated cells have preserved a memory or stem-cell-like phenotype so critical to improving the efficacy of ACT for cancer or viral infection.

Nishimura et al. provide additional insight into whether terminally differentiated $CD8^+$ T cells can be rejuvenated through reprogramming by showing that redifferentiated $CD8^+$ T cells have

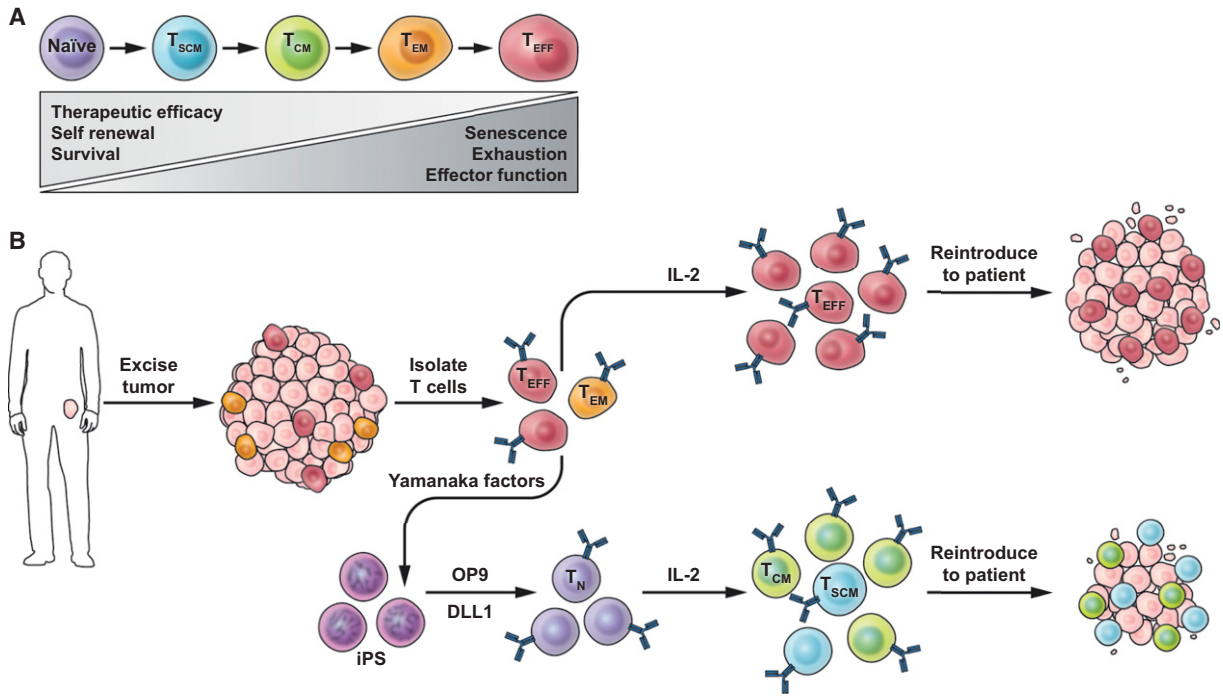

Figure 1. Reprogramming an Exhausted Immune System for Treatment of Cancer

(A) Generalized model of T cell differentiation. An immune response is initiated when naive $CD8^+$ T cells (T_N) are activated by their cognate antigen to undergo clonal expansion and differentiation into subsets characterized here by a reciprocally graded capacity for self-renewal and cytolytic effector function. Although eradication of tumor ultimately requires robust cytolytic function of terminally differentiated effector (T_{EFF}) cells, therapeutic efficacy of ACT is improved by transfer of less-differentiated cells because of their ability for self renewal and survival and their ability to generate T_{EFF} progeny.

(B) Rejuvenating T cells with Yamanaka factors. Adoptive T-cell-based immunotherapy (ACT) for cancer involves isolating $CD8^+$ lymphocytes from a surgically excised tumor and expanding the cells ex vivo with T cell growth factor IL-2. Lymphocytes are subsequently infused back into the patient to attack the remaining tumor sites. Lymphocytes isolated from the tumor generally have a terminally differentiated effector (T_{EFF}) or effector memory (T_{EM}) phenotype, and the IL-2 driven expansion results in further differentiation. There is considerable evidence that transfer of less-differentiated lymphocytes such as stem-cell memory T cells (T_{SCM}) or central memory T cells (T_{CM}) results in substantially greater antitumor immunity. Therefore, rejuvenating lymphocytes to a less-differentiated state using induced pluripotent stem cell (iPSC) technology shows great promise in substantially improving the efficacy of ACT. Reprogramming with Yamanaka factors and redifferentiation with Delta-like 1 expressing OP9 (OP9/DLL1) stromal cells rejuvenates lymphocytes and preserves less-differentiated T_{SCM} and T_{CM} $CD8^+$ T cell subsets that may result in improved eradication of tumor when transferred into cancer patients.

elongated telomeres and greater proliferative capacity. Using an HIV-1-epitope specific clone of $CD8^+$ T cells, they demonstrate reprogramming through an iPSC intermediate into functional $CD8^+$ T cells capable of cytolytic activity and interferon gamma release with recognition of their cognate peptide. Importantly, the cells also show enhanced expression of CCR7, CD27, and CD28, revealing characteristic stem-cell-like traits compared to the original T cell clone from which the cells were derived (Gattinoni et al., 2011). In fact, reincarnated $CD8^+$ T cells have even longer telomeres than their original counterparts, perhaps imparted by their sojourn through the iPSC state where telomerase activity runs hot. The authors duly recognize that the clinical translation of rejuvenating cytotoxic T cells to treat patients with HIV is complicated, and with good reason, but we feel this remains a remarkable demonstration of rejuvenated T cells and is an important first step in broadening the therapeutic application of ACT to treat maladies whose common denominator is a terminally differentiated and exhausted immune response.

Taken together, the work of the two groups led by Vizcardo and Nishimura demonstrate that antigen-experienced $CD8^+$ T cells can be reincarnated through an iPSC intermediate into $CD8^+$ T cells that preserve memory for their original epitope, and moreover, develop a rejuvenated memory phenotype upon redifferentiation. This has important clinical implications for ACT because it provides proof of concept that terminally differentiated lymphocytes can be reincarnated from an exhausted state to a memory phenotype that may have demonstrably superior antitumor immunity (Figure 1). We look forward to further preclinical work investigating the efficacy of reprogrammed T cells in eradicating tumors and chronic viral diseases and feel this may be a particularly fruitful means to hasten the bench-to-bedside translation of iPSC technology and regenerative medicine for the treatment of patients with viral-associated diseases and cancer.

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Making Healthy Stem Cells: The New Role of TPO

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Thrombopoietin (TPO) attracts much attention as an effective stimulus for blood cell formation in patients with hematopoietic disorders. In this issue of *Cell Stem Cell*, de Laval et al. (2013) show that TPO can also promote "healthy" hematopoietic stem cells when administered before radiotherapy to minimize HSC injury and mutagenesis in mice.

Thrombopoietin (TPO) has a well-described role as a principal regulator of platelet production, which is stimulated through binding to its receptor Mpl on megakaryocytes. More recently, studies have revealed important roles for TPO/Mpl signaling in hematopoietic stem cells (HSCs) as well (reviewed in Chou and Mulloy, 2011). Mpl is expressed in HSCs and progenitors, and TPO cooperates with other cytokines to promote expansion of HSCs in culture. Mice deficient in TPO and Mpl signaling have reduced numbers of HSCs with impaired repopulating ability as well as the defective platelet formation one would expect. Clinically, inactivating mutations of Mpl in humans cause thrombocytopenia and multilineage marrow failure, while activating Mpl mutations are involved in myeloproliferative disorders. These observations demonstrate an important role of TPO/Mpl signaling in HSCs and progenitors. As a mechanism for TPO-mediated HSC regulation, it was shown that TPO regulates HSC quiescence and interaction with the osteoblastic niche (Qian et al., 2007; Yoshihara et al., 2007).

Cells in the human body are continually exposed to DNA stresses. Physiological stresses as well as environmental agents, such as ionizing radiation (IR) and other genotoxic chemicals, induce DNA damage including double-strand breaks (DSBs). DNA repair is essential for cell survival, and the long lifespan of HSCs suggests that they need an effective DNA repair process to maintain a "healthy" state. DNA damage is repaired through two main pathways: homologous recombination (HR) and nonhomologous end joining (NHEJ). Recent data showed that quiescent HSCs preferentially use NHEJ repair mechanism, and DSB repair through NHEJ is necessary for HSC maintenance (Mohrin et al., 2010; Nijnik et al., 2007; Rossi et al., 2007).

In this issue of *Cell Stem Cell*, de Laval et al. (2013) discovered a new function of TPO in DNA damage response in HSCs and progenitors. They found that Mpl-deficient HSCs and progenitors had significantly increased numbers of γH2AX foci (a marker of DSB formation) after exposure to IR or topoisomerase-II inhibitors relative to wild-type cells. A neutral comet assay (another technique for the detection of DSBs) also showed IR-induced DNA damage was greatly enhanced in Mpl-deficient cells. Consistent with these findings, HSCs treated in the absence of TPO showed similar DSB repair defects and, conversely, TPO injection into mice just before IR reduced the number of γH2AX in HSCs in vivo. No differences in cell cycle and apoptotic status of HSCs were apparent under these experimental settings, implying the TPO/Mpl signaling was affecting DNA damage in HSCs through a direct effect on the DNA repair process. Other hematopoietic cytokines such as SCF and FLT3 ligand did not show such effects, suggesting that the DNA repair activity is a specific function of TPO. de Laval et al. (2013) found that TPO increased phosphorylation of the DNA-PK catalytic subunit (a central player in NHEJ), and pharmacological or genetic inhibition of DNA-PK abolished TPO-mediated DNA repair. TPO did not increase IR-induced Rad51 foci formation nor did it improve repair of DSBs induced by replicative stress, indicating HR was not involved in this DSB repair. Taken together, these results indicate that TPO/Mpl promotes NHEJ-mediated DNA repair by stimulating DNA-PK activity in HSCs. Importantly, TPO treatment before IR limits IR-induced HSC injury and increases HSC function for long-term hematopoietic reconstitution, raising the potential clinical application of TPO agonists in patients receiving radiotherapy or chemotherapy. The findings also imply that agonists of TPO/mpl may be effective in patients suffering from inefficient hematopoiesis caused by damaged HSCs.